

Supplemental Figure Captions

Figure S1. *In vitro* kinase reaction yields quantitative phosphorylation of accessible Cdk consensus sites.

A kinase reaction was carried out as described in the Methods section with purified, recombinant Rb⁷⁷¹⁻⁹²⁸ as a substrate. This construct contains seven Cdk consensus sites lacks intrinsic structure such that the Cdk S/TP consensus sites are presumed accessible. After the kinase reaction, phosRb⁷⁷¹⁻⁹²⁸ was purified with reverse phase HPLC. The single peak in the chromatogram was analyzed with electrospray ionization mass spectrometry. The spectrum of unphosphorylated Rb⁷⁷¹⁻⁹²⁸ was also taken for comparison (black). Deconvolution of the spectrum corresponding to phosRb⁷⁷¹⁻⁹²⁸ (red) corresponds to a single dominant species with a mass that is 560 Da (equivalent to seven additional phosphates) greater than the mass of Rb⁷⁷¹⁻⁹²⁸.

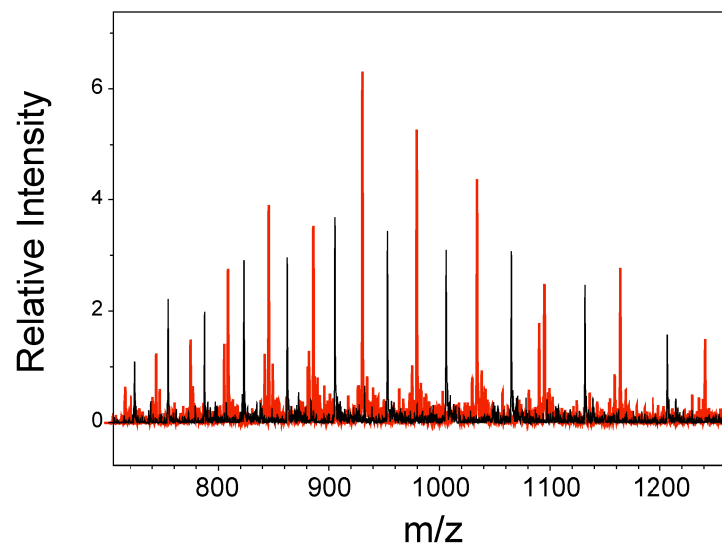
Figure S2. Sample isothermal titration calorimetry data for binding assays

Raw data and isotherms for binding reactions as indicated.

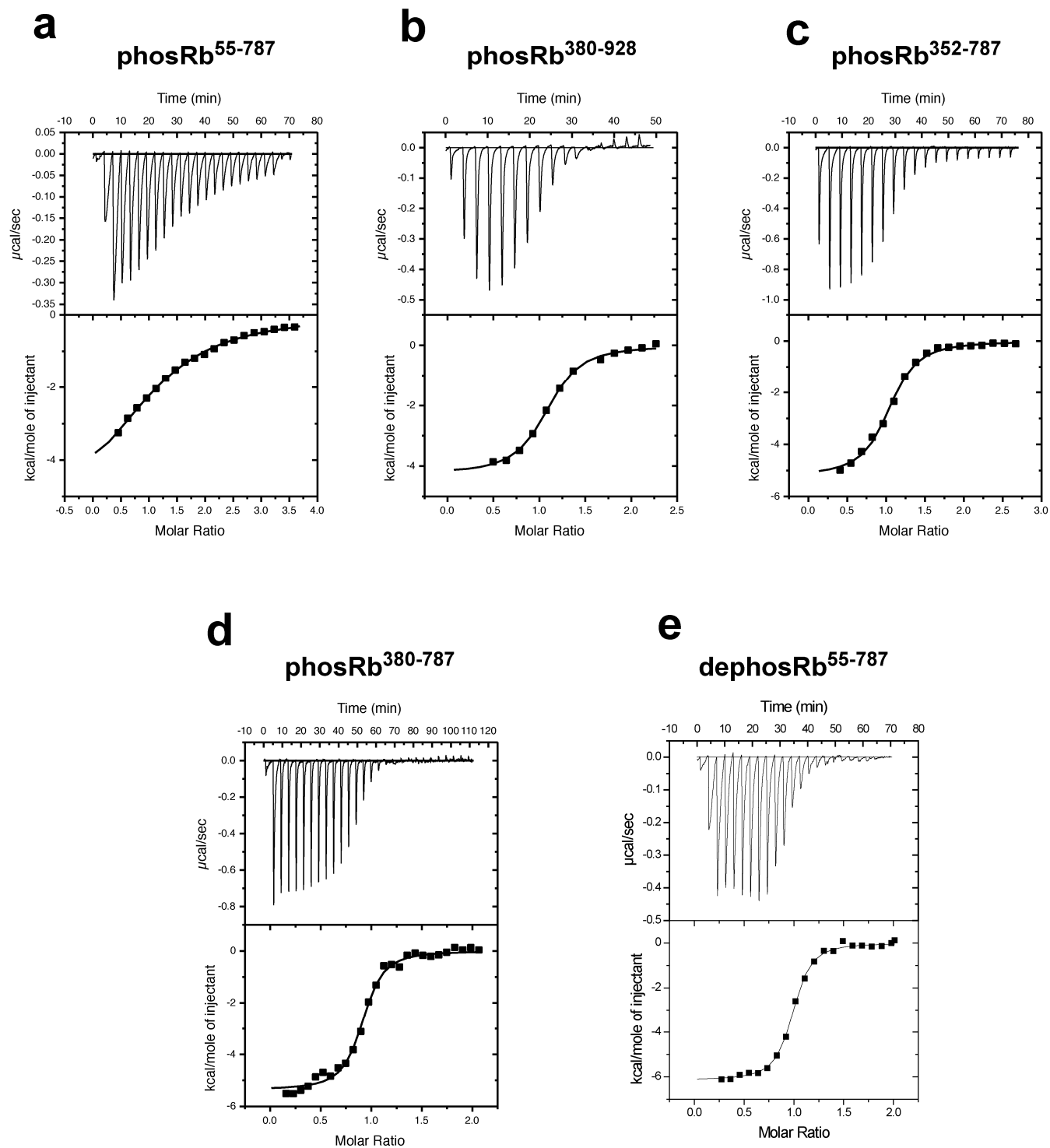
Figure S3. Full HSQC spectra of RbP^{APL}

300 μ M ²H-¹⁵N-labeled RbP^{APL} alone (black) and in the presence (red) of unlabeled (A) phosRbN-RbIDL (400 μ M), and (B) phosRbPL (1.5mM), and (C) E2F^{TD} (2 mM).

Supplemental Figure 1

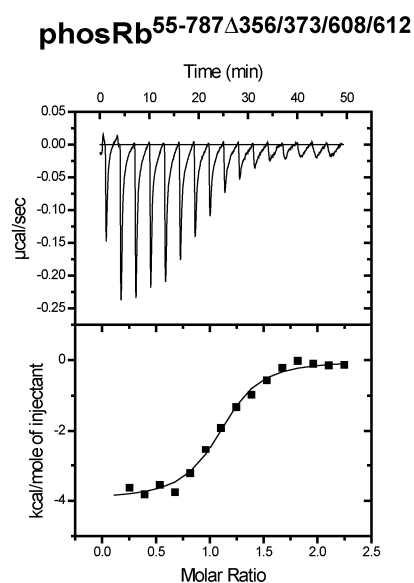


Supplemental Figure 2

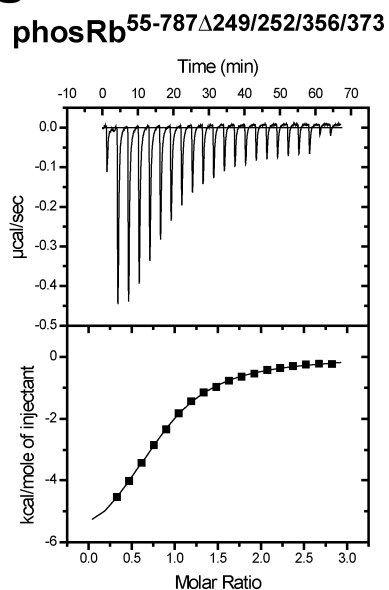


Supplemental Figure 2

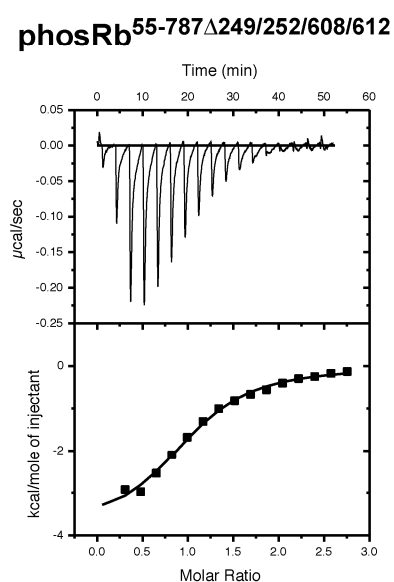
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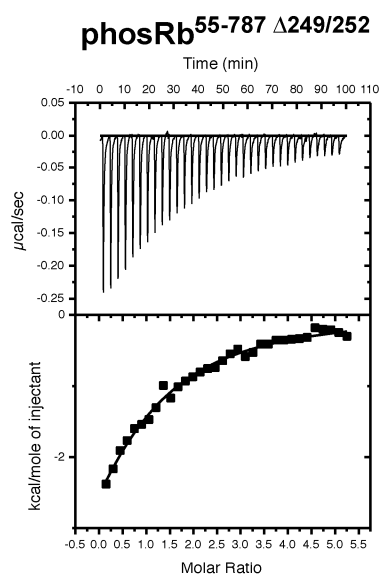
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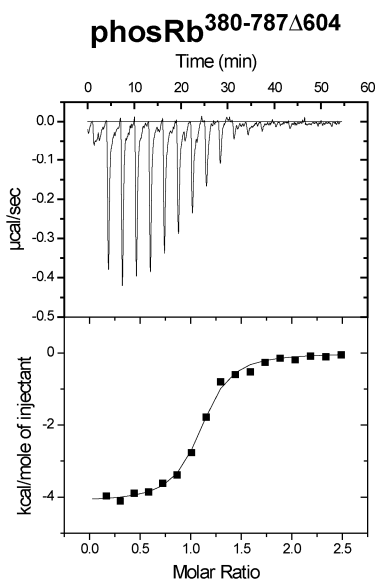
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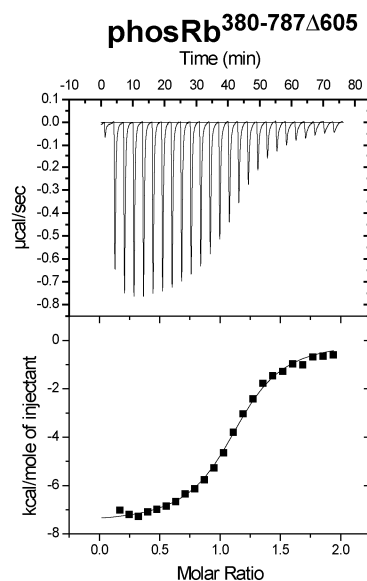
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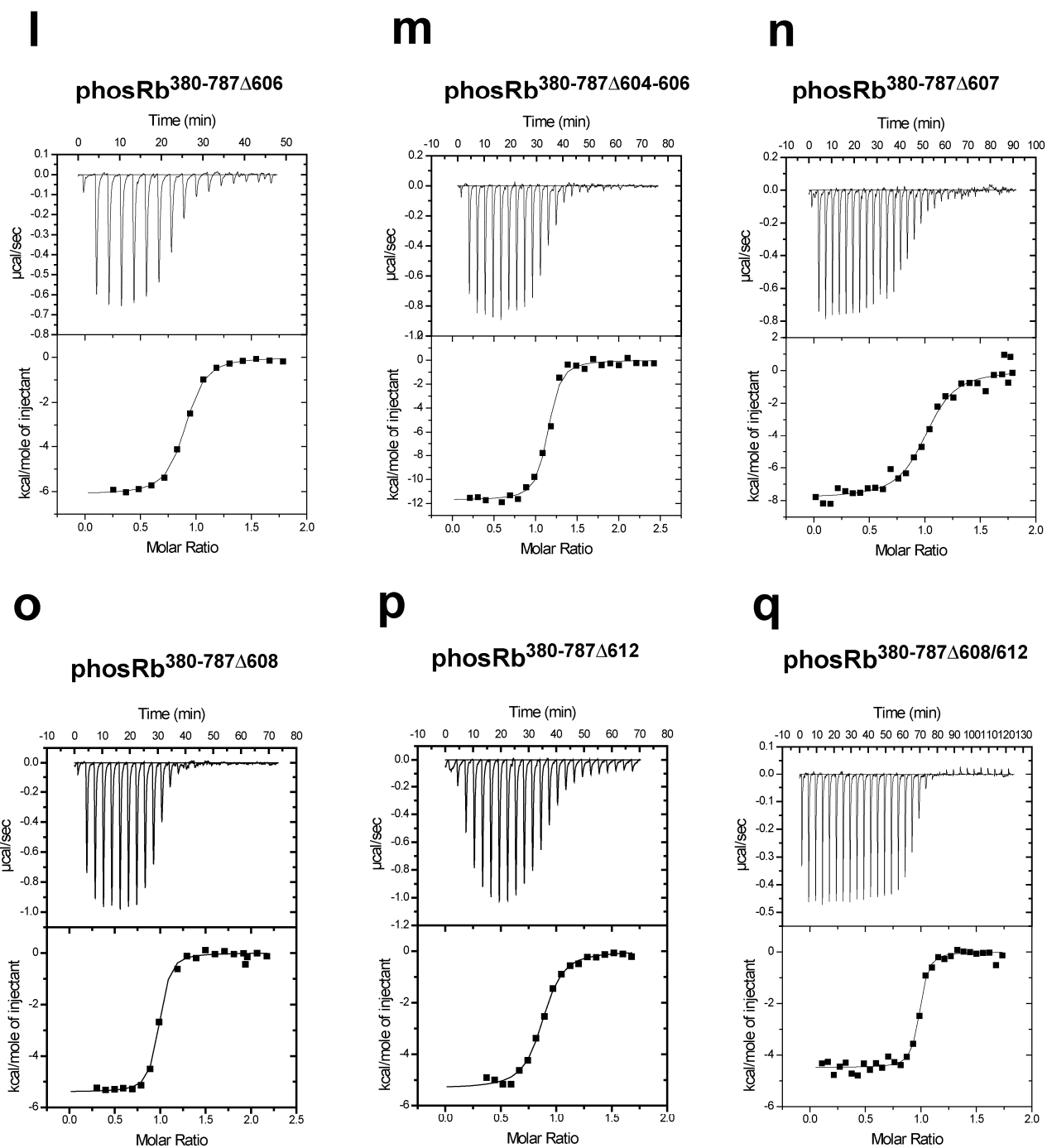
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Supplemental Figure 2



Supplemental Figure 3

